SELECTIVE RECOGNITION OF BOVINE SERUM ALBUMIN (BSA) USING MOLECULAR IMPRINTING POLYMER TECHNIQUE

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ABSTRACT

The aim for this research is to produces protein separation tool by a new technique to separate protein molecule from protein solution. The technique used is the molecularly imprinted polymer, MIP. In this technique the target molecule that we want to separate will be the template and it mixed with selected monomers. Through polymerization process the template molecule will trapped between the polymer matrix that become solid and stable in term of heat and chemical. To make it has high selectivity on protein molecule the solid polymer is pounded to fine particles and through hydrolysis process the template is extracted and will leave complementary sequences that have exact configuration like the template shape. And further, two type of study were carried out, the first was analysis of particles structure in characterization of particles by using FT-IR spectrum and the second was study on the performances of this technique by conducting three experiments. The first experiment was the adsorption of BSA protein in MIP or synthesized polymer particles, the second experiment was the effect of protein concentration on separation and third experiment was to observe the effect of particles mass on separation. Polymerization and hydrolysis was successfully occur and it shown by the FT-IR spectrum. Experiment show that adsorption of protein was high for MIP particle that was 52.5 % while adsorption for NIP particle was 33.0 %. For the effect of concentration and mass of particle, it shows that the availability particle is the main factor that influence the amount of substrate separated

***Keywords*:** Molecular imprinting polymer; separation; BSA protein

ABSTRAK

Kajian ini menumpukan pada penghasilan satu kaedah baru dalam pemisahan molekul-molekul protein dalam larutan protein. Kaedah yang digunakan adalah teknik penandaan molekul pada polimer. Teknik ini merupakan satu teknik dimana target molekul yang hendak dipisahkan dijadikan sebagai acuan dan acuan ini dicampurkan dengan monomer terpilih. Melalui proses polimerisasi molekul yang terlibat akan terperangkap diantara jaringan-jaringan polymer yang mengeras menjadi pepejal yang stabil keatas haba dan kimia. Pepejal polimer yang terhasil dipecahkan kepada partikel-partikel halus dan seterusnya menjalani proses hydrolisis. Dalam proses ini molekul protein yang terperangkap diekstrak dan meninggalkan kawasan aktif yang mempunyai bentuk yang serupa dengan bentuk molekul protein tersebut. Selanjutnya dua kajian dibuat, yang pertama mengkaji struktur partikel dengan spectrum FT-IR dan yang kedua mengkaji persembahan teknik ini melalui tiga eksperimen. Pertama adalah eksperimen pemisahan molekul protein BSA dengan partikel polimer, eksperimen kedua, kebolehan tindakan pemisahan terhadap kepekatan protein yang berlainan dan kebolehan tindakan pemisahan terhadap protein pada jisim partikel yang berbeza. Proses polimerisasi dan hydrolysis berlaku dengan jayanya dan dibuktikan melalui spectrum FT-IR. Daripada hasil experiment, penderapan protein BSA dengan menggunakan penandaan molekul pada polimer adalah tinggi iaitu 52.5%, manakala penderapan protein untuk partikel tanpa penandaan pada polimer adalah 33.0 %. Untuk eksperimen kesan kepekatan dan jisim partikel, keputusan eksperimen menunjukkan kuantiti partikel menjadi factor utama mempengaruhi kadar pemisahan substrat yang dikehendaki.

***Kata kunci*:** Penandaan molekul pada polimer; pemisahan; protein BSA

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AIBN	-	2, 20-Azobisisobutyronitrile (AIBN).
cAMP	-	cyclic Adenosine Monophospate
CSF	-	Cerebrospinal Fluid
bio	-	Biology, Biological
BSA	-	Bovine Serum Albumin
DMPAP	-	Dimethyl Phenyl Acetophenone
FT-IR	-	Fourier Transform Infra-red
M	-	Molar
EGDMA	-	Ethylene Glycol Dimethacrylic
MAA	-	Methacrylic Acid
MIP	-	Molecular Imprinting Polymer
NIP	-	Non-Imprinted Polymer
PCR	-	Polymerase chain reaction
PVC	-	Polyvinyl chloride
SDS-PAGE	-	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SPE	-	Solid Phase Extraction
uv	-	Ultra violet
^{o}C	-	Degree celcius
%	-	Percentage
µM	-	microMolar

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Date : .....

UNIVERSITI MALAYSIA PAHANG

BORANG PENGESAHAN STATUS TESIS♦

JUDUL : SELECTIVE RECOGNITION OF BOVINE SERUM ALBUMIN (BSA) USING MOLECULAR IMPRINTING POLYMER TECHNIQUE

SESI PENGAJIAN : 2009/2010

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CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Molecular imprinting polymer is now an established method for the production of synthetic receptors that can be functional in separation that can be applied in separation procedures and chemical analyses (C. Alexander *et al.,* 2003). The technique involves the formation of complexes between a print molecule (template) and a functional monomer based on relatively weak, noncovalent interactions (hydrogen bonding, ionic, hydrophobic, etc.) (C. Alexander *et al.,* 2003). These complexes appear spontaneously in the liquid phase and are then fixed sterically by polymerization with high degree of cross-linking (Mosbach *et al.,* 1999). After extracting the print molecules from the synthesized polymer, free recognition site, which are able to recognize the template during subsequent rebinding procedures, remain in the polymer matrix (Fenxia Qiao *et al.,* 2006). Chemically and mechanically stable molecularly imprinted polymer (MIP) able to recognize specific substances may successfully serve as substitutes for antibodies, enzymes or other native biological structures in fundamental investigations of molecular recognition and may have numerous other applications in biotechnology, medicine, environmental control, etc.

The combination of molecularly imprinted polymer (MIP) and solid phase extraction is reviewed. Solid-phase extraction (SPE) is one of the major application

fields for molecularly imprinted polymers (MIP) (Fenxia Qiao *et al.*, 2006). MIP, which has special features in term of selectivity as state above, has been used as sorbents for SPE to selectively isolate target molecule from it solution.

In proteins separations antibodies is one of the most precise and highly recognize toward the targets proteins. It has specific selectivity based on the type of proteins that want to separate. This MIP technique resembles the function of antibody. The MIPs synthesized in this study is tested for it ability to selectively recognize the target molecules BSA protein in protein solution.

1.2 PROBLEM STATEMENT

Separation process is important in science nature. In chemistry and chemical engineering, a separation process is used to transform a mixture of substances into two or more distinct products. The separated products could differ in chemical properties or some physical property, such as size, or crystal modification or other separation into different components. In this study it is about to develop a separation tool of protein, Bovine Serum Albumin by using a new technique called MIP. In separation processes that involve protein, most method used is expensive and the separation process is only can be done in a little volume of protein solution. This developed MIP for protein separation is will be the preferred for protein separation as it is cheaper with simple preparation and rapid mass of separation can be done (Fenxia Qiao *et al.,* 2006). Molecular imprinting polymer (MIP) has developed rapidly during the past three decades as an emerging fabrication strategy that yields nano structured assemblies possessing molecular recognition capabilities cited as references (Fenxia Qiao *et al.*,2006). MIP can produce material which high capability and high selective synthetic receptors for a variety of chemical and biochemical structures (Phil Brown *et al.,* 2008). The approach in this study is to develop a smart material having recognition ability to capture the protein, bovine serum albumin (BSA) by using this technique.

1.3 OBJECTIVE OF RESEARCH

The main objectives of this research are to synthesis MIP for bovine serum albumin protein (BSA) recognition and to study the performance of protein BSA separation using this MIP technique.

1.4 SCOPES OF RESEARCH

In order to achieve the objective in this research, scope of study was divided into two as the following:

i) To synthesis BSA-MIP particles.

ii) To study the performance of MIP for protein BSA separation.

CHAPTER 2

LITERATURE REVIEW

2.1 MOLECULAR IMPRINTING POLYMER AN INTRODUCTION AND APPLICATION

Molecular recognition is the basic concept in nature. Pauling et al (1940) was the first person to discuss the lock and key mechanism to explain molecular recognition as cited (Phil Brown *et al.*, 2008). The concept of molecular imprinting based on interaction is very old but their applications in various fields are emerging recently for sensor applications (Theodoridis *et al.,* 2003). Within the last five years in particular, interest in the area has surged, and it is now estimated that well over 100 academic and industrial research groups are active world-wide (Radha Gupta *et al.*,2008). To date, more than 500 articles and reviews describing molecular imprinting research have appeared in open literature and a significant number of patents are held in the area (Figure 2.2).

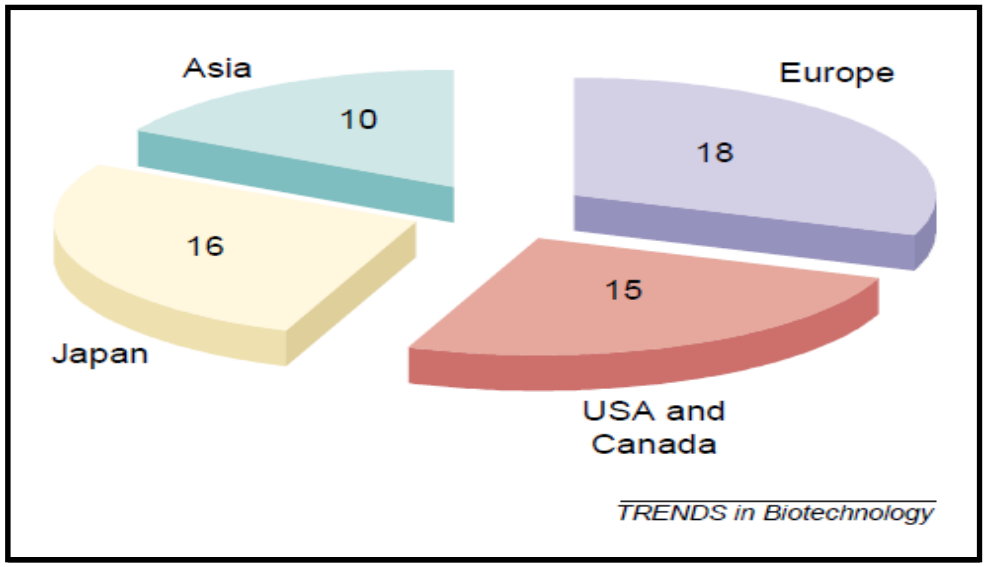


Figure 2.1: Number of research groups actively working with MIP

* Sergey A.Piletsky *et al.*, (2001) (Source: TRENDS in biotechnology)

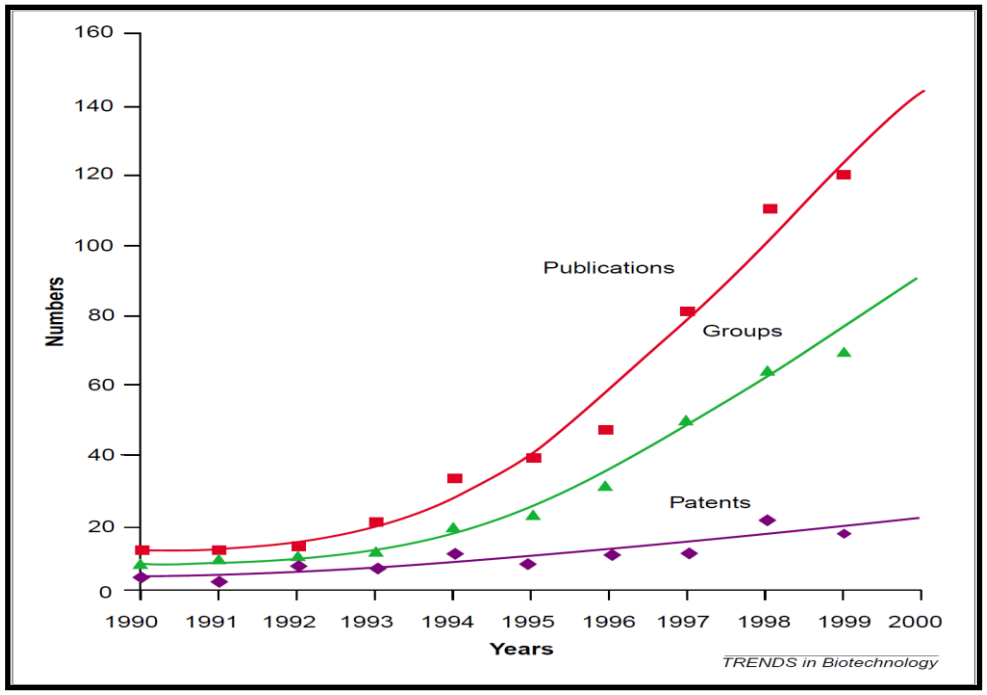


Figure 2.2: Progress in the development of molecular imprinted polymers

* Sergey A.Piletsky *et al.*, (2001) (Source: TRENDS in biotechnology)

The first examples of molecular imprinting by use of artificial organic polymers were independently reported by Kiefer et al., in 1972 and Wulff and Sarhan as cited in (Phil Brown *et al.*, 2008) and have since then found applications in separation processes (Chromatography, capillary electrophoresis, solid phase extraction (SPE), membrane separation), micro reactors, immunoassay and antibody mimics, catalysis and artificial enzymes, biosensor recognition elements and bio- and chemo-sensors (Phil Brown *et al.*,2008).

Table 2.1: Typical examples of MIPs design and application

Templates	Application	References
Amino Acids and Amino Acid Derivatives	Separation and binding synthesis, Assays and Sensors	Theodoridis *et al.*, Ramaier N *et al.*,
Aniline, Phenol and their Derivatives	Assays and Sensors	Phil Brown *et al.*, K. Takeda *et al.*,
Drug	Separation and binding, Assays and Sensors	A.L.Hillberg *at el.*,
Gases and Vapors		Theodoridis *et al.*,
Herbicides	Separation and binding, Assays and Sensors	Jun Matsui *et al*
Heterocycles	Separation and binding	Piletsky *et al.*,
Metal Ions	Separation and binding, Assays and Sensors	C. Baggiani *et al.*,
Nucleic acid and nucleic acid derivatives	Separation and binding, Assays and Sensors	Piletsky *et al.*,
Polynuclear aromatic hydrocarbon	Separation and binding, Assays and Sensors	Piletsky *et al.*,
Proteins	Separation and binding	Huntington *et al.*,
Steroids	Separation and binding, Detection	Theodoridis *et al.*,
Sugar and Sugar derivatives	Separation and binding, Assays and Sensors	Piletsky *et al.*,
Alkaloids, toxins and narcotics	Separation and binding, Assays and Sensors	Jun Matsui *et al.*,
Cell, viruses	Recognition and binding	Peter Kofinas *et al.*,

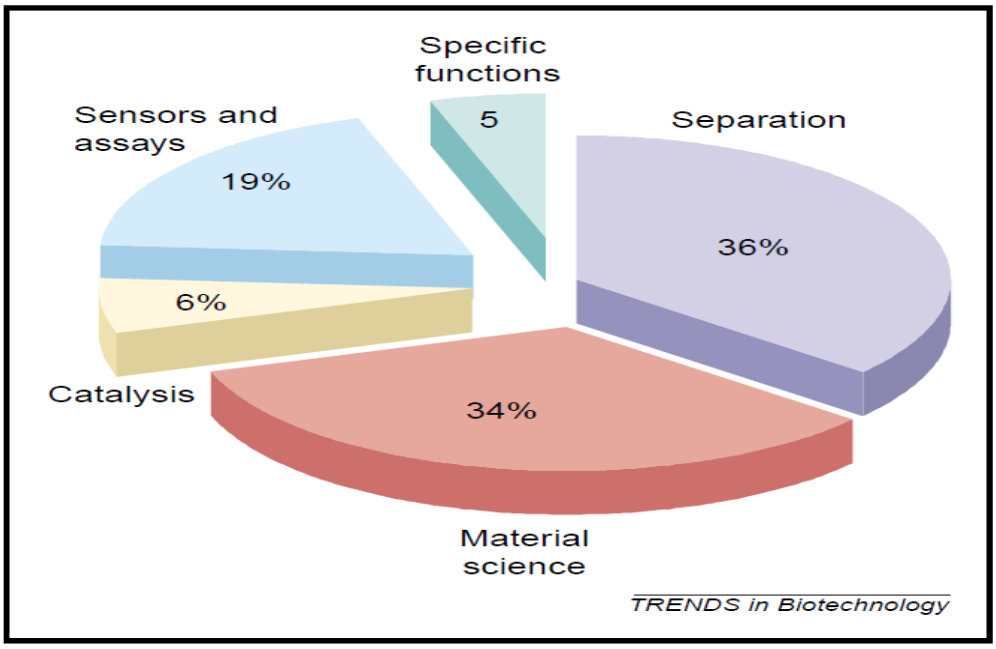
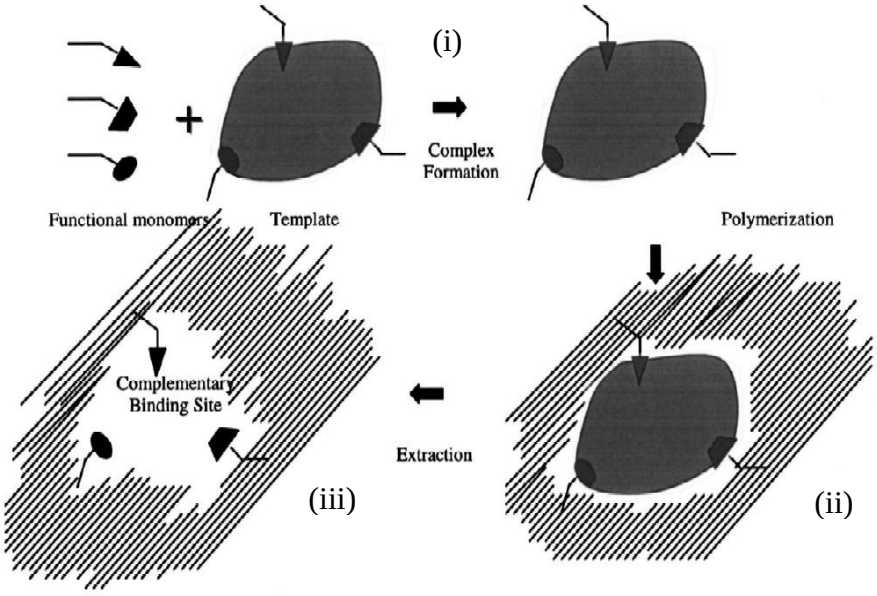


Figure 2.3: Schematic representation of research area in MIP technology in 1999. *Sergey A.Piletsky *et al.*, (2001) (Source: TRENDS in biotechnology)

The field of molecularly imprinted polymers (MIP) will evolve further to include new applications such as recognition elements in intelligent drug delivery devices, in targeted drug delivery applications and in micro fluids devices with application as analyte sensing micro-valves and micro actuators (Phil Brown *et al.*, 2008).

The molecular imprinting technique can be applied to different kinds of target molecules, ranging from small organic molecules (e.g. pharmaceuticals, pesticides, amino acids and peptides, nucleotide bases, steroids and sugar) to polypeptides, high molecular proteins and even whole cells (R. Narayanaswamy *et al.*,).



Scheme 2.1: Illustration of synthesizing of molecular imprinting polymer

*Petr Bures *et al.*, (2001) (Source: Journal of controlled release)

The principle of molecular imprinting is shown in (Scheme 2.1). Generally the fabrication of MIPs consist of three main steps (i) A self-assembled complex is formed between a template and suitable monomers, Initially a noncovalent prepolymerisation complex is formed in solution (ii) after the addition of a crosslinker, polymerization initiated, resulting in the immobilization of the template within the complex, (iii) template extraction leaves a molecularly imprinted polymer (MIP) with specific reciprocal recognition sites for the template molecule. The MIP produce is stable in various chemical and physical conditions for a long time and can be reused without any alteration to the cavity formed (Phil Brown *et al.*, 2008).

The size and shape of the cavity allow the target molecule or similar molecule to occupy the cavity space (Alexandre Rachkov *et al.*,2000). While the functional group orientation within the cavity will preferentially bind in specific locations to only the target molecule and to similar molecule (Piletsky *et al.*, 2001). This make this technique has greater recognition capability compare to others method (Jun Matsui *et al.*, 2000).

2.2 PROTEIN

Proteins are highly complex molecules which contain the elements of carbon, hydrogen, nitrogen, and usually sulfur or sulphur. They are synthesized by living cells and are an essential part of the structure of the cell and its nucleus. The plant proteins are more easily isolated in crystalline form. Proteins are stored in plants in the form of aleurone grains. They are required for animals as the source of nitrogen in food.

Protein —> Polypeptide -4 Peptide —> Amino acids

Since proteins are present in all living organisms, they are of great importance in biochemistry. They form an important class of food. Example meat, fish and egg are important source of animal proteins. Cereal grains, example wheat, pulses, and etc are plant protein foods. Whole glandular products, oil-bearing plant seeds, antitoxins, serums, and globulins contain proteins in combination with other biochemical substances. These products possess therapeutic activity. Allergens are usually proteinaceous materials producing allergic reactions.

Certain proteins are highly poisonous. Among them are plant toxalbumins, ricin from castor beans, robin from locus: bark, abrin from jequirity seeds, hemolysins from salamanders and various toxins, e.g. neurotoxins from snake venom.

2.2.1 ALBUMIN PROTEIN

The most well-known type of albumin is serum albumin. It is most common in the blood or serum (providing its name) but it can also appear in other fluid compartments (providing the basis for the cerebrospinal fluid (CSF)/serum albumin ratio, for example.) Serum albumin is the most abundant blood plasma protein and is produced in the liver and forms a large proportion of all plasma protein. The human

version is human serum albumin, and it normally constitutes about 60% of human plasma protein.

Generally albumin play significant role in human and animal body. Low albumin (hypoalbuminemia) may be caused by liver disease, nephrotic syndrome, burns, protein-losing enteropathy, malabsorption, malnutrition, late pregnancy, artefact, genetic variations and malignancy. High albumin (hyperalbuminemia) is almost always caused by dehydration. In some cases of retinol (Vitamin A) deficiency the albumin level can become raised to High-normal values (ex: 4.9 g/dL). This is because retinol causes cells to swell with water (this is also the reason too much Vitamin A is toxic). In lab experiments it has been shown that All-trans retinoic acid down regulates human albumin production.

Specific types include in albumin proteins:

- Human Serum Albumin

- Bovine Serum Albumin (cattle serum albumin) or BSA, often used in medical and molecular biology labs

- Bovine Serum Albumin (chicken serum albumin)